

Post-transcriptional regulation in higher eukaryotes: The role of the reporter gene in controlling expression

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Summary. We have investigated whether reporter genes influence cytoplasmic regulation of gene expression in tobacco and Chinese hamster ovary (CHO) cells. Two genes, *uidA* encoding β -glucuronidase (GUS) from *Escherichia coli* and *Luc*, encoding firefly luciferase (LUC), were used to analyze the ability of a cap, polyadenylated tail, and the 5'- and 3'-untranslated regions (UTR) from tobacco mosaic virus (TMV) to regulate expression. The regulation associated with the 5' cap structure and the TMV 5'-UTR, both of which enhance translational efficiency, was reporter gene-independent. The poly(A) tail and the TMV 3'-UTR, which is functionally equivalent to a poly(A) tail, increase translational efficiency as well as mRNA stability. The regulation associated with these 3' ends was highly reporter gene-dependent; their effect on GUS expression was almost an order of magnitude greater than that on LUC expression. In tobacco, the tenfold reporter gene effect on poly(A) tail or TMV 3'-UTR function could not be explained by a differential impact on mRNA stability; GUS and LUC mRNA half-life increased only twofold when either the poly(A) tail or TMV 3'-UTR was present. In CHO cells, however, GUS mRNA was stabilized to a greater extent by a poly(A) tail or the TMV 3'-UTR than was LUC mRNA.

Key words: mRNA stability – Translational efficiency – Cap – Poly(A) tail – Higher eukaryotes

Introduction

In recent years, there has been an increase in the number and type of examples demonstrating the importance of

post-transcriptional regulation in controlling gene expression. mRNA sequences involved in regulating expression include those within a pre-mRNA involved in splicing (Buchman and Berg 1988; Callis et al. 1987a) or nucleocytoplasmic transport (Malim et al. 1989); those within the 3' untranslated region (UTR) involved in message stability (Brawerman 1981, 1987; Casey et al. 1988; Jones and Cole 1987; Levine et al. 1987; Mullner and Kuhn 1988; Pandey and Marzluff 1987; Shaw and Kamen 1986; Wilson and Treisman 1988; Wreschner and Rechavi 1988) or within the 5'-UTR involved in translational efficiency (Aziz and Munro 1987; Cullen 1988; Gallie et al. 1989, 1987b; Geballe et al. 1986; Jobling and Gehrke 1987; Logan and Shenk 1984; Mueller and Hinnebusch 1986; Pelletier and Sonenberg 1988; Werner et al. 1987; Williams et al. 1988); those surrounding an AUG codon involved in initiation codon selection (Gallie et al. 1987b; Kozak 1987; Lutcke et al. 1987); and those within the coding region involved in establishing the rate of ribosome translocation (Wolin and Walter 1988). The role of the cap and the poly(A) tail as mRNA determinants essential for efficient expression has been well documented (for reviews see Brawerman 1981, 1987; Rhoads 1988).

We have shown that the tobacco mosaic virus (TMV) leader, called Ω (Mandeles 1968), substantially enhances translation of Ω -containing reporter gene constructs in plant protoplasts of both host and non-host species (Gallie et al. 1989, 1987b) and, to a much lesser extent, in Chinese hamster ovary (CHO) cells (Gallie and Walbot 1990). Ω -associated regulation is not a result of increased message stability, but is a function of ribosome concentration and type (Gallie et al. 1988).

Recently, we demonstrated that the non-polyadenylated 3'-UTR of TMV is the functional equivalent of a poly(A) tail in the cytoplasm and both a poly(A) tail and the 3'-UTR of TMV substantially increase gene expression in higher eukaryotes compared to mRNAs lacking either mRNA element (Gallie and Walbot 1990). The entire TMV 3'-UTR is involved in extensive higher order interactions and is composed of two domains: a

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tRNA-like structure that forms the 3' terminus of the viral RNA and a domain entirely comprised of RNA pseudoknots (van Belkum et al. 1985). This latter domain is largely responsible for the regulation associated with this region.

Although most eukaryotic mRNAs are polyadenylated, there are exceptions, such as the cell-cycle regulated histone mRNAs (Levine et al. 1987; Pandey and Marzluff 1987), in which a stem-loop structure located within the 3'-UTR regulates turnover. All plant mRNAs analyzed thus far, including histone messages, are polyadenylated; several RNA viruses terminate in a highly structured domain similar to TMV (Hall 1979; Haenni et al. 1982).

In this study, we examined the role of the reporter gene as a modulator of mRNA features known to regulate gene expression. We investigated two 5' determinants, a cap and the TMV 5'-UTR, and two 3' determinants, a poly(A) tail and the TMV 3'-UTR. The β -glucuronidase (GUS) gene (*uidA*) and luciferase (LUC) gene (*Luc*) were used as the reporter genes. Using T7-based vectors, pairs of mRNAs containing identical determinants but differing in the reporter gene were synthesized *in vitro*. Tobacco protoplasts or CHO cells were transfected with these mRNAs using electroporation and the impact of the reporter gene on the function of a cap, TMV 5'-UTR, poly(A) tail, or TMV 3'-UTR was determined *in vivo*.

Materials and methods

Plasmid constructs. The Ω and poly(A)-containing GUS (Jefferson et al. 1986) constructs have been described previously (Gallie et al. 1989). The TMV 3'-UTR-containing GUS constructs have also been described (Gallie and Walbot 1990). The corresponding LUC constructs were made from each equivalent GUS construct by first excising the *uidA* gene using *SalI* and replacing it with the *Luc* gene as a *SalI* fragment. The mRNAs produced from these constructs are illustrated in Fig. 1.

***In vitro* transcription.** The T7-based GUS and LUC constructs were linearized with the appropriate restriction enzyme, and *in vitro* transcription of the DNA was carried out using bacteriophage T7 RNA polymerase (Schenborn and Mierendorf 1985). Capped transcripts were synthesized by modifying the published reaction conditions to include 200 μ M GTP and 1.5 mM $m^7G(5')ppp(5')G$ (New England BioLabs). RNA integrity was determined by formaldehyde-agarose gel electrophoresis (Melton et al. 1984).

Preparation and electroporation of plant protoplasts. Protoplast media and isolation methods for cell suspensions of tobacco (Xanthi) have been described (Fromm et al. 1987) except that 1.0% Cytolase (Genencor) was used in place of 0.5% Rhozyme. The cells were digested for approximately 1.5 h before harvesting. Electroporation conditions for tobacco were 180 V, 8 msec pulse, 1550 μ F capacitor using an X-cell TM 450 Electroporation System (Promega Biotec). mRNA was added to 1×10^6 protoplasts followed by immediate electroporation on ice. After 10 min, the protoplasts were removed from the ice and allowed to recover for 18 h before collection for analysis. Greater than 90% of the protoplasts are reproducibly transformed by this procedure (Gallie et al. 1989).

Preparation and electroporation of CHO cells. CHO cells were cultured in Ham's F12 medium (Gibco) containing 10% fetal bovine serum and were released from the plate by trypsinization. The cells were washed once in phosphate-buffered saline and used immediately. Standard electroporation conditions for the CHO cells were 450 V and 250 μ F capacitor using a Bio-Rad Gene Pulser. Electroporation of 5×10^6 cells was carried out at room temperature and, after 10 min, the cells were added to fresh Ham's F12 serum-supplemented medium, and allowed to recover for 18 h. Error associated with RNA electroporation is $\pm 15\%$.

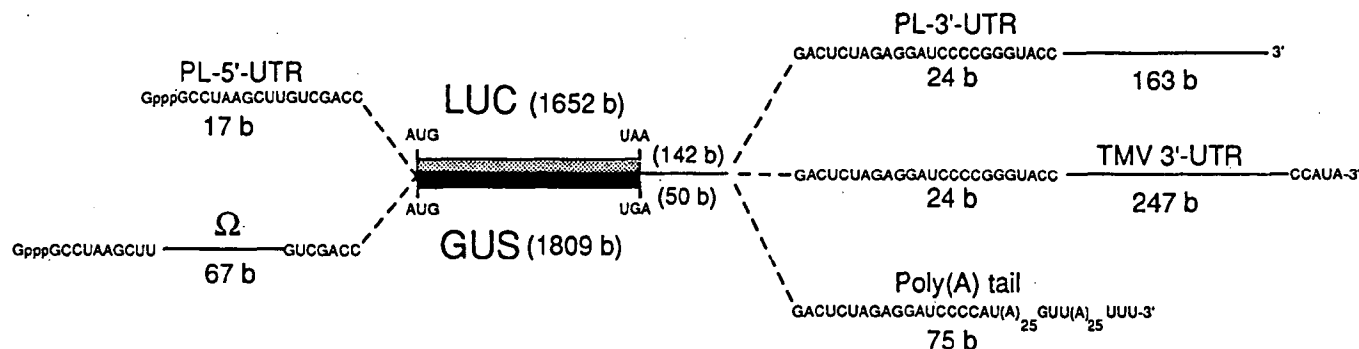


Fig. 1. mRNA construct for analysis of Ω , the tobacco mosaic virus 3'-untranslated region (TMV 3'-UTR), and a poly(A) tail in tobacco and Chinese hamster ovary (CHO) cells. All constructs contain precisely the same *uidA* or *Luc* gene (not drawn to scale). The *Luc* gene contains 135 bases of the native 3'-UTR (without the poly(A) tail); the *uidA* gene contains 43 bases of the native 3'-UTR. Two 5'-UTRs were used: the 67 base TMV 5'-UTR (Ω)

and a 17 base polylinker sequence. Three 3'-UTRs were used: the TMV 3'-UTR (positions 6150–6396 of the TMV genome); a poly(A) tail of either 25 or 50 adenylate residues; and poly(A⁻) control sequence (PL-3'-UTR) derived from the modified pBluescript vector. The PL-3'-UTR, TMV 3'-UTR, and poly(A) tail sequences shown are those resulting from transcription of each vector when linearized with *BglI*, *NdeI*, and *DraI*, respectively

mRNA stability. Aliquots of tobacco protoplasts or CHO cells electroporated with 5 µg of each mRNA were removed at specific time intervals, the cells collected by centrifugation, washed once and total RNA extracted using the procedure of Chomczynski and Sacchi (1987). After resuspension, the RNA was denatured and loaded onto a formaldehyde-agarose gel, followed by Northern transfer to Nytran membrane (Schleicher and Schuell); the blots were probed with GUS or LUC anti-sense RNA. The region of the membrane representing the full-length form of the GUS mRNA was cut from the membrane, counted, and the log. of the values plotted against time. The slope of the best-fit line through the data points, k , was used to calculate the half-life for each construct according to the equation $t_{1/2} = 0.693/k$.

Analysis of GUS and LUC activity. Plant protoplasts or trypsinized CHO cells collected by centrifugation were resuspended and sonicated in 0.5 ml of buffer (50 mM sodium phosphate, pH 7.0, 10 mM β -mercaptoethanol, 1 mM EDTA). For GUS assays, 4-methylumbelliferyl- β -D-glucuronide (Sigma) was added to 1 mM to aliquots which were then incubated at 37°C for 15–120 min; the reaction was terminated by the addition of 0.2 M sodium carbonate. Fluorescence was measured by excitation at 365 nm and emission at 455 nm in a TKO 100 DNA Fluorometer (Hoefer Scientific Instruments). GUS specific activity was determined as nmol of substrate per min per mg of protein. Protein was determined using the Bio-Rad protein assay kit.

For LUC assays, 20 µl cell extract aliquots were added to 100 µl buffer (25 mM glycylglycine pH 7.8, 15 mM $MgCl_2$, 5 mM ATP, 7 mM β -mercaptoethanol, 1 mg/ml bovine serum albumin), to which 100 µl of 250 µM luciferin was injected; the photons produced in 10 s were measured using a Monolight 2001 Luminometer (Analytical Luminescence Laboratory).

Results

The impact of the reporter gene on the function of a cap and the TMV 5'-UTR

In our study of the interaction between the coding and non-coding regions of a messenger RNA in establishing the level of expression, we chose two reporter genes, the *uidA* and *Luc* genes, as model systems in which we could examine the impact of changes in the 5'- and 3'-UTRs. By employing reporter genes which are not normally present in plant or mammalian cells, we endeavored to uncover the general principles that underlie cytoplasmic regulation. Sequence changes however, were minor, representing no more than 15% of the total mRNA sequence and involved only non-coding regions. The unaltered sequence of the coding region remained, therefore, as a "functional unit", e.g. in assuming any secondary structure native to the coding region.

A complete set of *uidA* constructs containing either the TMV 5'-UTR (Ω) or a 17 base polylinker leader combined with either a poly(A) tail, the TMV 3'-UTR,

or vector-derived sequence (Fig. 1) had been made previously (Gallie and Walbot 1990). In order to make equivalent constructs containing the *Luc* gene (de Wet et al. 1987), the *uidA* gene in each construct was replaced with the *Luc* gene (Fig. 1).

We have demonstrated the importance of a cap for the efficient translation of GUS or chloramphenicol acetyltransferase (CAT) mRNAs in plant protoplasts and COS cells (Callis et al. 1987b; Gallie et al. 1989). To make a direct comparison of a cap's effect on GUS or LUC expression, 1 µg of Ω -GUS-poly(A)₂₅ and Ω -LUC-poly(A)₂₅ mRNA synthesized in capped or uncapped forms was electroporated into the same preparation of either tobacco protoplasts or CHO cells. In tobacco, the cap increased GUS expression 52-fold from 0.05 to 2.62 units and stimulated LUC expression to approximately the same degree, from 0.122×10^6 to 5.17×10^6 light units/mg protein (42-fold). In CHO cells, the presence of the cap resulted in a 136-fold increase in GUS expression (from 0.34 to 46.3 units) and a 150-fold increase in LUC expression (from 9.2×10^3 to 1450×10^3 light units/mg protein). An additional 19–48% increase was observed in either tobacco or CHO cells when the cap was monomethylated (m^7GpppG ; data not shown). The effect of a cap on expression, therefore, was unaffected by either reporter gene and exhibited a significant impact in both plant and animal cells.

The TMV 5'-UTR, Ω , can substantially increase translation in plants (Gallie et al. 1989) as well as in *Xenopus* oocytes (Gallie et al. 1987a, b); however, the Ω -associated enhancement in rabbit reticulocyte lysate is only 1.5-fold. To determine whether this response to Ω was equally low in vivo and to make a direct comparison between CHO cells and tobacco protoplasts, capped GUS-A₅₀ and capped Ω -GUS-A₅₀ mRNAs were divided into equal portions for electroporation into the

	Specific Activity (nmol/min mg)	
	TOBACCO	CHO CELLS
GpppG AUG GUS UGA PL-3'-UTR BamHI	0.01	1.36
GpppG AUG GUS UGA (A) ₂₅ BamHI	0.34	20.0
GpppG AUG GUS UGA (A) ₅₀ BamHI	0.71	29.9
GpppG AUG GUS UGA TMV-3'-UTR BamHI	0.60	30.5
GpppG Ω AUG GUS UGA PL-3'-UTR BamHI	0.29	4.42
GpppG Ω AUG GUS UGA (A) ₂₅ BamHI	10.2	44.1
GpppG Ω AUG GUS UGA (A) ₅₀ BamHI	15.2	60.8
GpppG Ω AUG GUS UGA TMV-3'-UTR BamHI	29.3	70.6

Fig. 2. Expression of β -glucuronidase (GUS) mRNA in tobacco and CHO cells electroporated with capped mRNAs. Constructs are diagrammed in Fig. 1. Except for the changes within the 5'- and 3'-UTRs, the GUS mRNA constructs are otherwise identical. GUS mRNA from the same batch of in vitro-synthesized mRNA was used for electroporation into both cell types

			Luciferase Activity light units (x10 ³)/mg protein	
			TOBACCO	CHO CELLS
GpppG	AUG LUC UAA PL-3'-UTR	BamHI	26	462
GpppG	AUG LUC UAA (A) ₂₅	BamHI	106	910
GpppG	AUG LUC UAA (A) ₅₀	BamHI	340	1360
GpppG	AUG LUC UAA TMV-3'-UTR	BamHI	228	1480
GpppG	Ω AUG LUC UAA PL-3'-UTR	BamHI	746	572
GpppG	Ω AUG LUC UAA (A) ₂₅	BamHI	5210	1000
GpppG	Ω AUG LUC UAA (A) ₅₀	BamHI	12200	1630
GpppG	Ω AUG LUC UAA TMV-3'-UTR	BamHI	17100	1560

Fig. 3. Luciferase (LUC) expression in tobacco and CHO cells from capped mRNAs. Constructs are as diagrammed in Fig. 1. Except for the changes within the 5'- and 3'-UTRs, the LUC mRNA constructs are otherwise identical. LUC mRNA from the same batch of in vitro-synthesized mRNA was used for electroporation into both cell types

two cell types. When tobacco protoplasts were electroporated and assayed (Fig. 2), expression increased 21-fold as a result of the presence of Ω, while CHO cells showed only a 2.0-fold increase. Similar levels of enhancement by Ω were observed for the GUS constructs terminating in a poly(A)₂₅, TMV 3'-UTR, or vector-derived sequence. Thus, the difference in response to Ω by mammalian and plant cells is not the result of differences in mRNA preparations.

By examining parallel LUC constructs, we could ascertain whether Ω functions independently of the gene. The stimulation of LUC expression from the poly(A)₅₀ construct due to the presence of Ω remained approximately equivalent to that observed for the GUS constructs: 36-fold in tobacco and 1.4-fold in CHO cells (Fig. 3) Ω had a similar effect on the other LUC constructs. The impact of Ω on expression, therefore, is relatively unaffected by the gene.

The impact of the reporter gene on the function of a poly(A) tail and the TMV 3'-UTR

Virtually all eukaryotic nuclear-encoded mRNAs terminate in a poly(A) tail, suggesting that this ubiquitous mRNA feature is required for efficient expression in both animal and plant cells. The poly(A) binding protein, responsible for mediating the effect of the poly(A) tail and an essential gene in yeast (Sachs et al. 1987), has a minimum binding site requirement of 12 adenylate residues and effectively covers 25 adenylate residues (Sachs et al. 1987). A poly(A)₂₅ tail is sufficient to increase expression in tobacco protoplasts (Gallie et al. 1989) and expression of a reporter gene in COS cells is stimulated by a poly(A)₇₀ tail (Callis et al. 1987b).

In order to determine whether the effect of the polyadenylated tail on expression is influenced by the upstream gene, capped GUS and LUC mRNA constructs with or without a poly(A) tail were assayed (for the exact sequence at the 3' terminus, see Fig. 1). The presence of a poly(A)₂₅ increased GUS expression in tobacco 34-fold relative to poly(A⁻) GUS control mRNA and 15-fold in CHO cells (Fig. 2). A poly(A)₅₀ tail increased GUS expression 71-fold in tobacco and 22-fold in CHO cells. The parallel LUC constructs, however, responded quite differently to the presence of a poly(A) tail. The poly(A)₂₅ tail stimulated LUC expression only 4.1-fold in tobacco and 2.0-fold in CHO cells. Likewise, the poly(A)₅₀ tail stimulated LUC expression 13-fold in tobacco and 2.9-fold in CHO cells. This is markedly less than the effect the poly(A) tail had on GUS expression. We conclude, therefore, that the upstream gene can play an important role in determining the degree to which a polyadenylated tail regulates expression.

Viral mRNAs, such as TMV RNA which functions as a messenger RNA as well as being the genomic RNA, represent the only known examples of non-polyadenylated mRNAs in plants discovered to date. The TMV 3'-UTR is functionally equivalent to a poly(A) tail in both plant and animal cells (Gallie and Walbot 1990). Analysis of the reporter gene effect on a second 3'-UTR regulatory determinant would therefore demonstrate whether the effect was limited to the polyadenylated tail or might be a general effect on downstream regulatory elements. GUS and LUC mRNA constructs terminating in the TMV 3'-UTR were therefore tested in both cell types.

As with a poly(A) tail, the regulation associated with the TMV 3'-UTR was markedly influenced by the upstream gene (Figs. 2, 3). The TMV 3'-UTR increased GUS expression (using the GUS construct terminating in the vector-derived sequence as a control) 60-fold in tobacco and 22-fold in CHO cells, whereas in the parallel LUC mRNA constructs, the TMV 3'-UTR increased expression only 8.8-fold in tobacco and 3.2-fold in CHO cells. Thus, for both downstream regulatory determinants, the degree to which they control expression is modulated by the upstream gene.

The effect of the 5' and 3' determinants on mRNA stability

The presence of a poly(A)₅₀ tail or the TMV 3'-UTR increased the stability of GUS mRNA 2- to 3-fold in tobacco cells (Gallie et al. 1989; Gallie and Walbot 1990). This stabilizing effect only partly explains the 50-100 fold increase in gene expression observed when either 3'-determinant is present in a GUS mRNA construct. This suggests that the TMV 3'-UTR and poly(A) tail largely affect translational efficiency. In order to ascertain whether the reporter gene effect was exerted at the level of message stability, we examined the half-life of GUS and LUC mRNA constructs terminating in either a poly(A)₅₀ tail, the TMV 3'-UTR, or the poly(A⁻) sequence. In tobacco protoplasts, the presence of a poly(A)₅₀ tail or the TMV 3'-UTR increased GUS mRNA

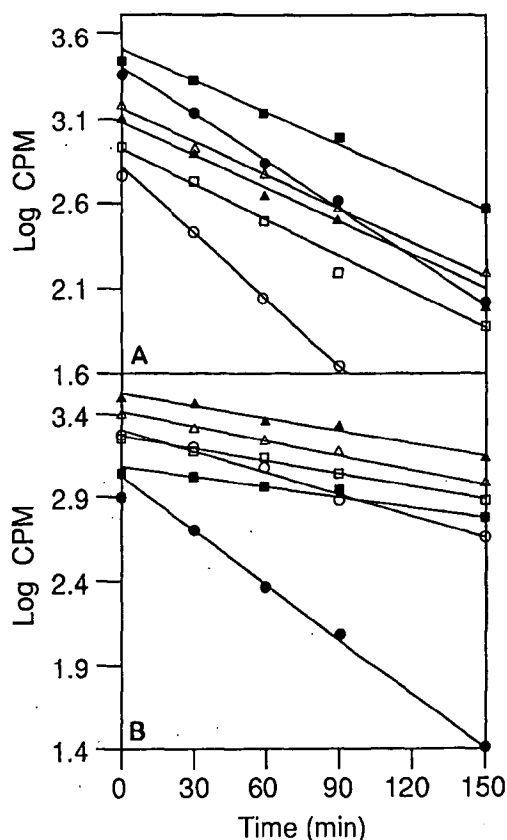


Fig. 4A and B. mRNA half-life determination in tobacco and CHO cells. The decay curve for each construct in electroporated tobacco (A) or CHO (B) cells is displayed as a log function of intact mRNA remaining over time. ●, Ω -GUS mRNA; ○, Ω -LUC mRNA; ■, Ω -GUS- A_{50} mRNA; □, Ω -LUC- A_{50} mRNA; ▲, Ω -GUS-TMV 3'-UTR mRNA; △, Ω -LUC-TMV 3'-UTR mRNA

half-life from 22 min to 53 or 47 min, respectively, and increased the half-life of LUC mRNA from 24 min to 43 or 45 min, respectively (Fig. 4A). As the stabilizing effect of the 3' determinants was approximately equivalent for the GUS and LUC mRNAs, the modulating effect of the reporter gene on 3' determinant regulation cannot be explained at the level of differential mRNA stability in tobacco cells.

In CHO cells, however, the presence of a poly(A)₅₀ tail or the TMV 3'-UTR increased the GUS mRNA half-life from 27 min to 150 or 111 min, respectively but only increased the LUC mRNA half-life from 67 min to 115 or 102 min (Fig. 4B). Therefore, the 3' determinants have a greater stabilizing effect on GUS mRNA (4- to 5.5-fold) than on LUC mRNA (1.5- to 1.7-fold) in CHO cells, partially accounting for the differential impact the 3' determinants have on GUS and LUC expression in animal cells. It is interesting to note that the control LUC mRNA was inherently more stable ($t_{1/2}$ = 67 min) than the control GUS mRNA ($t_{1/2}$ = 27 min) in CHO cells and inherently more stable in CHO ($t_{1/2}$ = 67 min) versus tobacco cells ($t_{1/2}$ = 24 min). It is possible that mRNA from the *Luc* gene, originally from firefly, is stabilized by factors conserved between firefly and mammals that are not present in plants.

Discussion

We have analyzed several mRNA determinants that affect expression post-transcriptionally, by regulating either translational efficiency or mRNA stability in animal and plant cells. The most general conclusion is that every structural component of an mRNA – the cap, the 5'-UTR, the coding region, the 3'-UTR, and the poly(A) tail – can potentially contribute to the cytoplasmic regulation of gene expression. This study has focused on how the individual determinants can affect expression independently of their original context and how they interact to modulate activity.

Those factors influencing translational efficiency in eukaryotes have recently become the subject of much investigation; the processes of both ribosome loading and initiation are implicated as important in the quantitative regulation of gene expression (reviewed in Rhoads 1988). A cap is essential for the efficient expression of reporter mRNAs electroporated into either plant or animal cells (Callis et al. 1987b; Gallie et al. 1989). Because the 40 S ribosomal subunit interacts with the cap binding complex and not with the cap itself (Rhoads 1988), the first contact the 40 S subunit makes with the mRNA is with the 5'-untranslated leader sequence; hence, leader structure or sequence provides an opportunity for regulation.

In this and prior work (Gallie et al. 1987b, 1989) we have demonstrated that the leader from TMV, Ω , can dramatically increase the efficiency of translation in plant species of mRNAs with or without a poly(A) tail. We demonstrate here that the results obtained from plant cells cannot be extrapolated to other eukaryotes as Ω increased expression much less in CHO cells. Secondary structure within the untranslated leader can be detrimental to the ribosomal scanning process in animal cells (Kozak 1986; Pelletier and Sonenberg 1985), however Ω lacks secondary structure. Although Ω may present no impediment to scanning ribosomes, the fact that Ω does not enhance translation in all higher eukaryotes suggests that the lack of secondary structure per se is not the major mechanism of Ω function. Moreover, the GUS and LUC coding regions, making up the majority of each mRNA construct examined in this study, should largely determine the secondary structure of the message. When present in either reporter mRNA, Ω increased the translational efficiency to the same extent, and was unaffected by any structural differences that may exist between GUS and LUC mRNAs.

A poly(A) tail of just 25 adenylate residues is sufficient for function in both plant and animal cells. Increasing the tail length to 50 adenylate residues boosts expression only an additional 1.4- to 3.0-fold; this suggests that 25 adenylate residues allow efficient interaction with the poly(A) binding protein (PAB) in higher eukaryotes. In yeast, the PAB binding site size is only 12 adenylate residues with a packing density of one PAB molecule per 25 adenylate residues (Sachs et al. 1987). Although little is known about the PAB protein in plants, it is a highly conserved protein in fungi and animals (Sachs and Kornberg 1985), suggesting that its function is simi-

lar for diverse organisms. It is interesting to note, therefore, that the impact of a poly(A) tail was markedly less in the CHO cells than in tobacco. The addition of a poly(A)₅₀ tail to GUS mRNA increased expression 71-fold in plant protoplasts but only 22-fold in animal cells. Similar results were observed with the parallel LUC constructs. In tobacco, the poly(A)₅₀ tail increased LUC expression 13-fold, whereas in CHO cells the increase was only 3-fold. Moreover, expression from poly(A⁻) GUS mRNA was below the limit of detection in tobacco, whereas in CHO cells significant levels of expression were measured for the same GUS construct. Thus, the role of the poly(A) tail in the cytoplasmic regulation of gene expression may be less important for recruitment of the mRNA into polysomes in animals than in plants.

Although the cap, 5'-UTR, 3'-UTR, and poly(A) tail may all function as potential regulatory determinants in cytoplasmic regulation, the reporter gene can also play a role in determining translational efficiency and message stability. Both the poly(A) tail and the TMV 3'-UTR had less of an impact on LUC expression than on GUS. It should be noted that the GUS constructs contain only a portion of the bacterial 3'-UTR of the *uidA* gene, whereas the LUC constructs contain the complete firefly *Luc* 3'-UTR. The region in LUC mRNA responsible for modulating the function of the poly(A) tail and the TMV 3'-UTR may therefore be located in the 3'-UTR, the coding region, or both. How does the *Luc* gene modulate function of the 3' determinants? One possibility may be that the LUC mRNA contains a ribonuclease cleavage site which acts as the rate-determining step in degradation; adding a poly(A) tail or the TMV 3'-UTR to the mRNA therefore, would do less to stabilize LUC mRNA than it would GUS mRNA. Differences in the degree to which the 3' determinants affected GUS and LUC mRNA stability was observed in CHO cells but not at all in tobacco. Differential message stabilization, therefore, cannot fully account for the reporter gene effect. As both the poly(A) tail and the TMV 3'-UTR function largely in regulating translation efficiency (Gallie et al. 1989; Gallie and Walbot 1990), it is probable that the reporter gene modulates their function as translational regulators.

Our data suggest that mRNA determinants involved in cytoplasmic regulation do not always act in isolation, but function within the context of the particular message, acting directly or through interactions with other mRNA determinants to establish the translational efficiency as well as the stability of a message.

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